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Organization and Development of Reflecting Platelets in Iridophores of the Giant Clam, *Tridacna crocea* Lamarck

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ABSTRACT—Giant clams show brilliant coloration on the mantle. The color comes from iridophores which are distributed in the mesenchyme. Each iridophore contains thin reflecting platelets which are aligned uniformly in rows. The platelet is bound with a membrane and has a fine substructure with a 7 nm lattice. A flat cistern intervenes two neighbouring platelets to keep the interspace constant.

In developing iridoblast, the reflecting platelets are formed in a confined area around the nucleus. Various types of vacuoles representing transitional froms from the ER to a mature reflecting platelet are seen in the area. Golgi vesicles are involved in the platelet formation. They are incorporated onto the vacuolar membrane while accumulation of the dense reflecting substance takes place in the vacuolar lumen. The dense substance is condensed in the vacuoles. The vacuoles are then fashioned into thin rectangular platelets and aligned in rows to form an alternating reflecting surface. The intervening cistern is formed from vesicles which fuse with one another to become a flat, thin cistern.

INTRODUCTION

In molluscs, two types of iridophores (iridocytes) have been identified. The first type is the iridophore which contains small granular or vesicular organelles by which the incident light is split and sent backwards to effect the Tyndall phenomenon. This type of iridophore has been observed in the mantle of opisthobranchiate gastropods [1]. The second type is the iridophore which shows a color by reflection and interference through multilayered platelets arranged uniformly in the cytoplasm. Cells of the second type are observed in the skin of cephalopods [2, 3], and in the mantle tissue of some bivalved shells [4, 5]. Because of their poorly arranged platelets, some of these cells display less effective coloration and have been referred to as reflector cells [6].

Iridophores of the giant clam show a clear monochromatic coloration in various spectral ranges according to the anatomical location of the cell. These iridophores contain multiple rows of reflecting platelets, each of which is uniform in thickness [4, 7]. The precision with which the platelets are arranged is reflected in a narrow range of spectrum, which leads to the giant clam producing one of the prominent colorations among molluscs.

In spite of their high efficiency as chromatophores, no detailed studies have so far been reported on the structure and development of the clam iridophores. This study intends to show the ultrastructural organization and the devolopment of reflecting platelets in iridophores of the giant clam.

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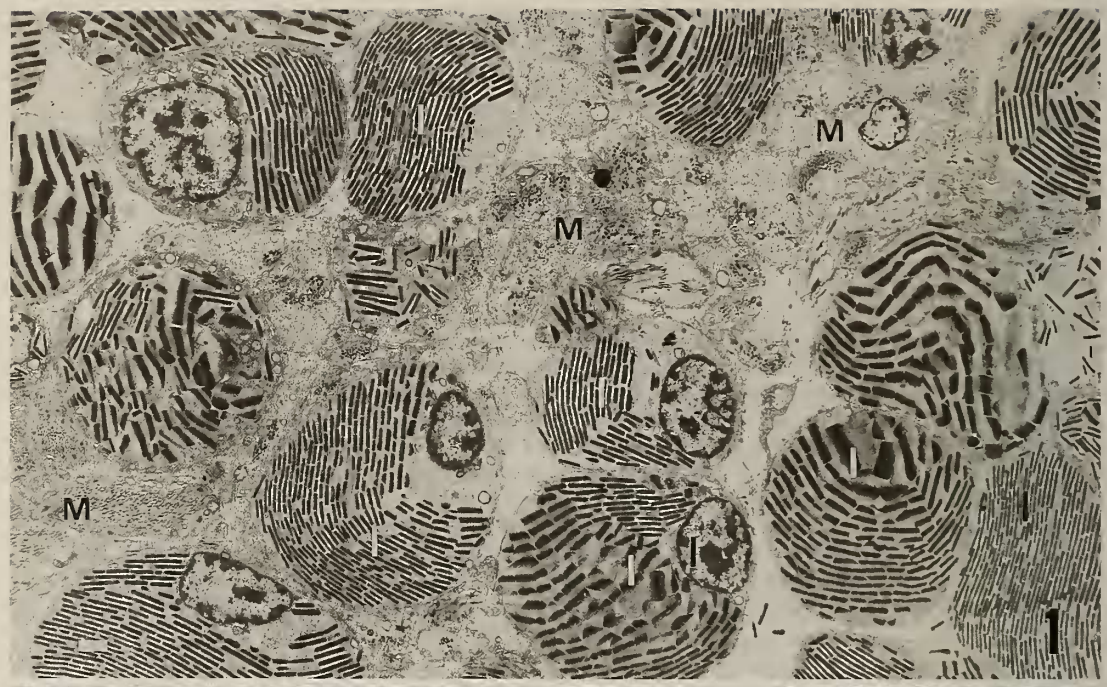
MATERIALS AND METHODS

Giant clams (*Tridacna crocea*) were collected from the Ryukyu Islands, the south-western archipelago of Japan. The brilliantly colored portion of the mantle tissue was excised and minced into small blocks in 3% glutaraldehyde fixative buffered to pH=7.4 with 0.1 M phosphate solution. Tissue blocks were further fixed in the same solution at room temperature for 2 hr and then transferred into 1% osmium tetroxide solution buffered with 0.1 M phosphate to pH 7.4. After the post osmification for 1.5 hr in the solution at the room temperature, tissue blocks were dehydrated through the ethanol series and embedded in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epoxy resin. Thin sections obtained on the LKB ultramicrotome 4800 A or the Porter-Blum MT-I ultratome mounted with glass knives were examined under the Hitachi Electron Microsope HU-11E.

RESULTS

Morphology of the iridophore

Mantle iridophores of the giant clam were observed in the mesenchyme. They were distributed mostly in clusters among muscle cells and positioned to cover the outer layer of digestive glands in which zooxanthellae, the algal symbionts, were colonized (Fig. 1). The iridophore was spherical or oval in shape. A round nucleus was usually lacated in the peripheral cytoplasm and the rest of the cell was filled with rows of reflecting platelets (Fig. 2). Mitochondria, vesicles and ribosomes were observed around the nucleus. A small number of vesicles and ribosomes were also found in the interspaces between the platelets.

The reflecting platelets were rectangular in shape (Fig. 5). Thickness of the platelet was uniform within each cell(Fig. 2), although it differed from cell to cell, ranging from 80 nm to 120 nm. The platelet was enveloped with a single limiting membrane, which measured 7 nm in thickness and was slightly thinner than the plasma or ER membrane (Figs. 3 and 4). At the end of the platelet, vesicles and ribosomes were often seen closely associated with the platelet (Figs. 3~6). The reflecting body mass of the platelet was electron dense and had a substructure of fine lattice (Fig. 6). The lattice consisted of an alternate arrangement of dense and light lines (2.0 nm and 5.0 nm in thickness, respectively) at 7.0 nm intervals. There was a narrow marginal space of 3 nm between the envelope and the inner reflecting body mass (Fig. 6). All platelets in a mature iridophore faced one direction and were aligned in parallel rows with a set interval (Figs. 2~3). In each row, the platelets were arranged end to end, forming a broad reflecting plane (Fig. 5).

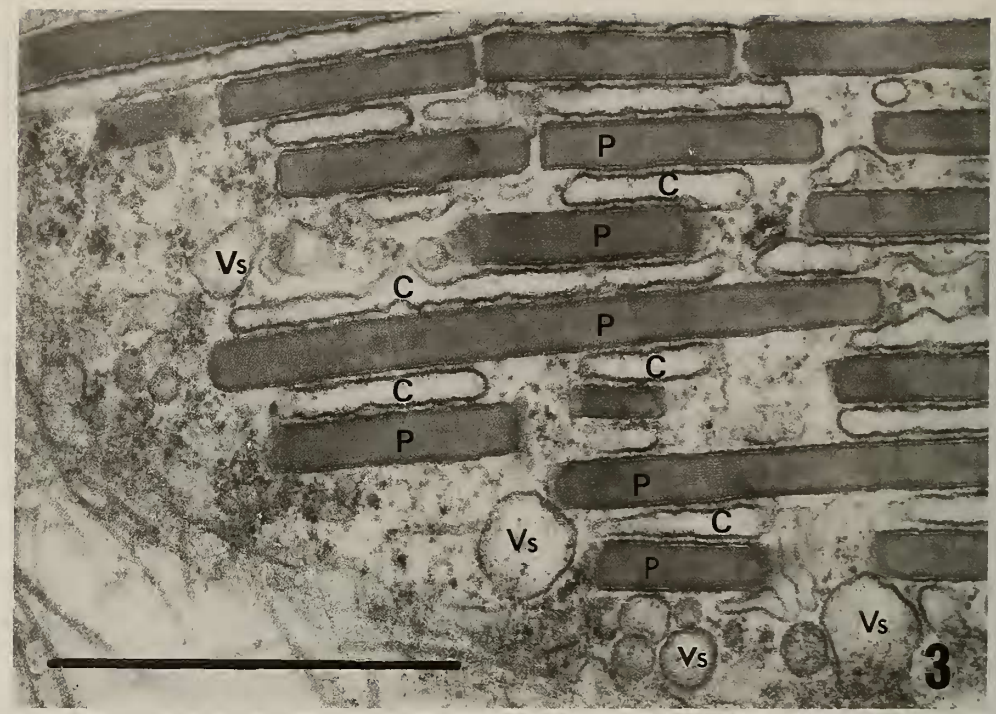
Flat and long cisterns lay between the platelet rows. The cistern was tightly secured between two platelets, ensuring a constant interspace (Figs. 2~4). The width of the cistern was uniform within each cell but differed slightly according to the cell. The width ranged from 50 nm to 60 nm in most cases, so that the distance between the dense reflecting masses of the platelets measured from 80 nm to 100 nm. The lumen of the cistern appeared to be empty, although a few granular substances were detected on the internal surface of the cisternal membrane. Cisterns were found only between the platelet rows, so that no cisterns were found along the outer side of the platelets at the extremity of the row (Figs. 3 and 11). Cisterns were often seen fusing with the cell membrane at the marginal end, so that their lumina were directly opened to the extracellular space (Fig. 4). The opening was always covered with the solid basal lamina which did not invaginate concomitantly with the plasma membrane.

Development of the iridoblast

In iridoblasts, especially in those in earlier developmental stages, many endoplasmic reticula and Golgi complexes were seen around the nucleus (Fig. 7). Numerous vacuoles and vesicles of various shapes and sizes were also observed in this area (Figs. 7~13). Developing platelets were observed in the vicinity of this area. The developing platelets were smaller than mature ones and often lacked the associating cisterns along them (Figs. 8, 11 and 12).

Fig. 1. Electron micrograph of the mantle tissue of a giant clam. Iridophores (I) are observed in clusters among musule cells (M). The iridophore is spherical and has a nucleus in the periphery. Most part of the cytoplasm is occupied with reflecting platelets. Each cell contains 20 to 30 rows of reflecting platelets which are aligned in parallel one another and are arranged around the nucleus. The orientation of the platelets differs in each cell. Bar indicates 1 μm. $\times$3,500

Fig. 2. Transverse profile of the platelet in a giant clam iridophore. Each row consists of a series of platelets (P) arranged end to end. Interspace between the platelet rows is kept in uniform distance by an intervening cistern (C). Only few organelles such as mitochondria, small vesicles and ribosomes are seen around the nucleus (N). Thick basal lamina (Bl) is seen outside of the iridophore plasma membrane (Pm). Bar indicates 1 μm. $\times$12,000



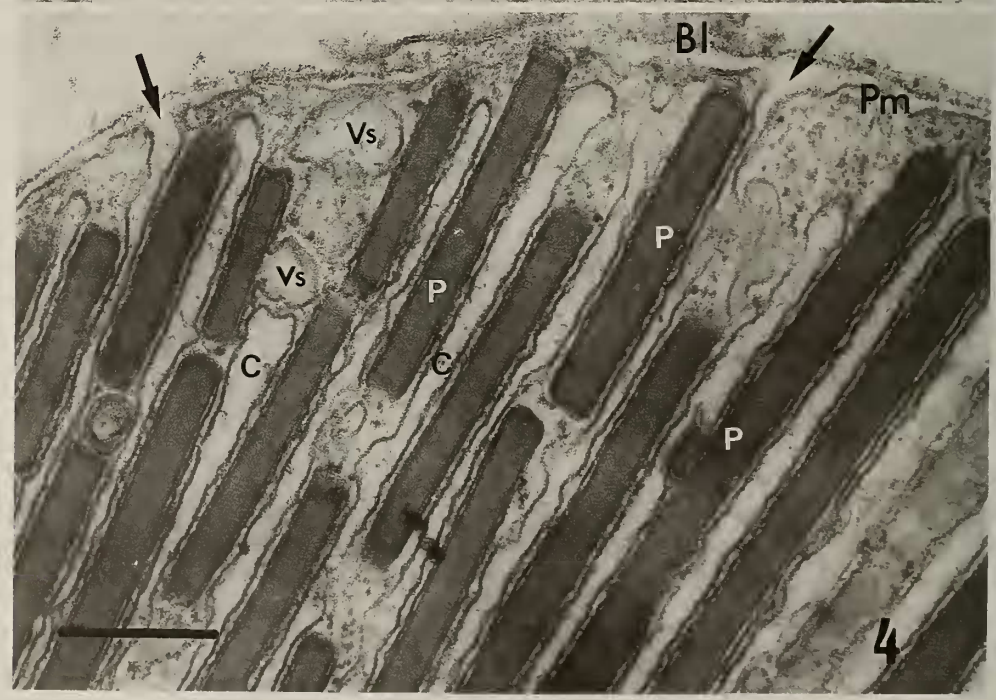
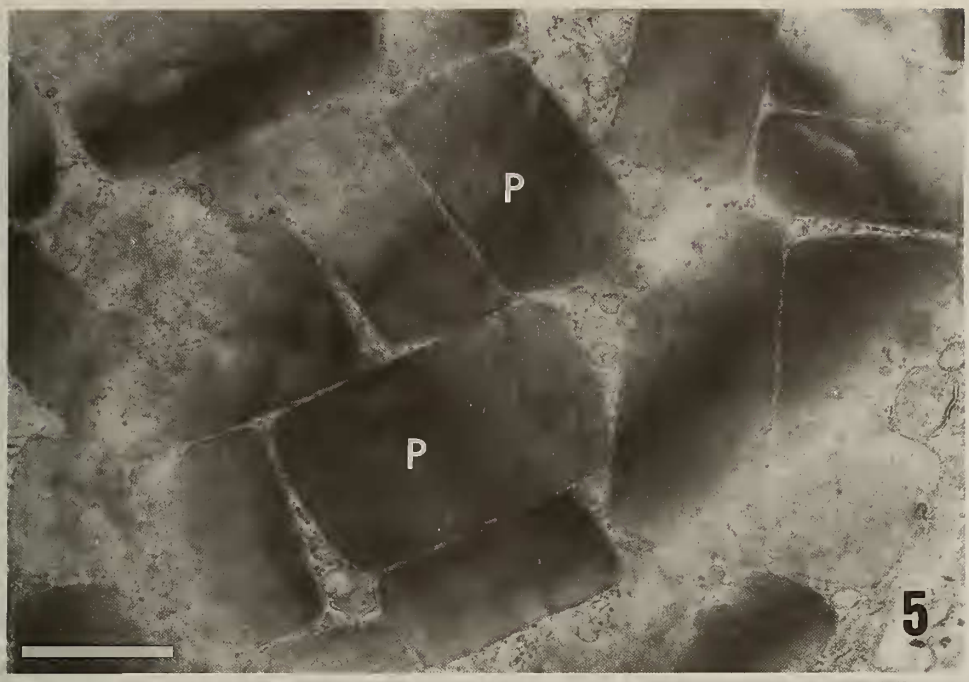


FIG. 3. A portion of platelet forming area near the cell surfaee showing developing eisterns. A flat eistern (C) with 50 nm to 60 nm width is tightly seeured between two platelets (P). No eisterns are seen along the outerside of the marginal platelets. Distended vesieles (Vs) are seen along the platelets. Ribosomes are seen among the platelets. Bar indicates 1 μ. $\times$54,000

FIG. 4. A peripheral portion of an iridophore seetioned transversely to the platelet row. Some of eisterns fuse with the plasma membrane at the periphery, so that the eisternal lumnen opens directly to the extracellular spaee (arrows). Note dilated vesieles (Vs) at the position of the cistern. Thiek basal lamina (Bl) is seen outside of the plasm membrane (Pm). Bar indieate 1 μm. $\times$43,700.



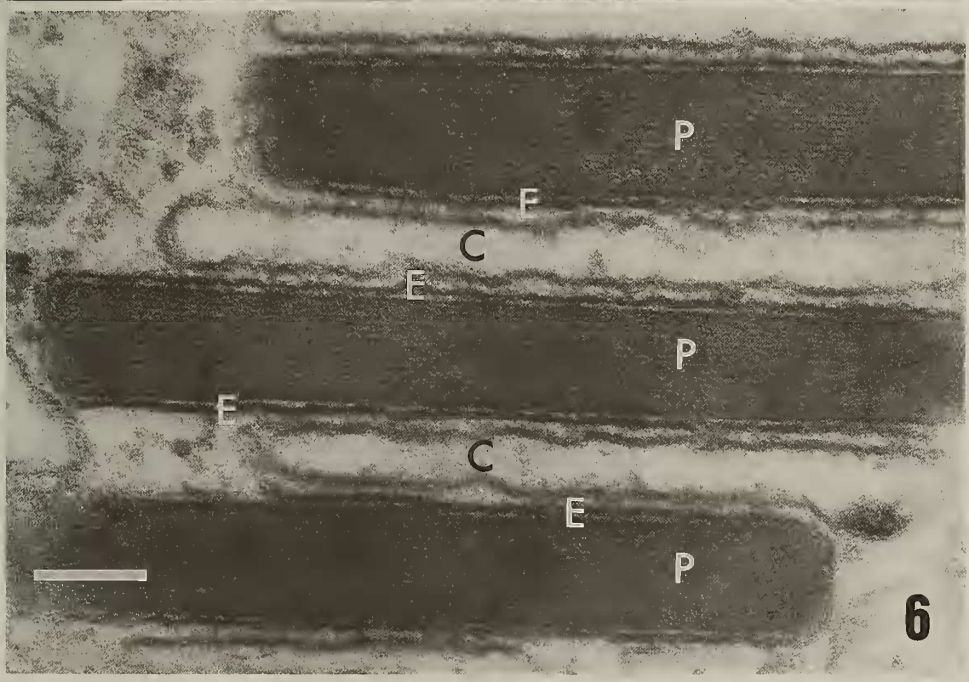
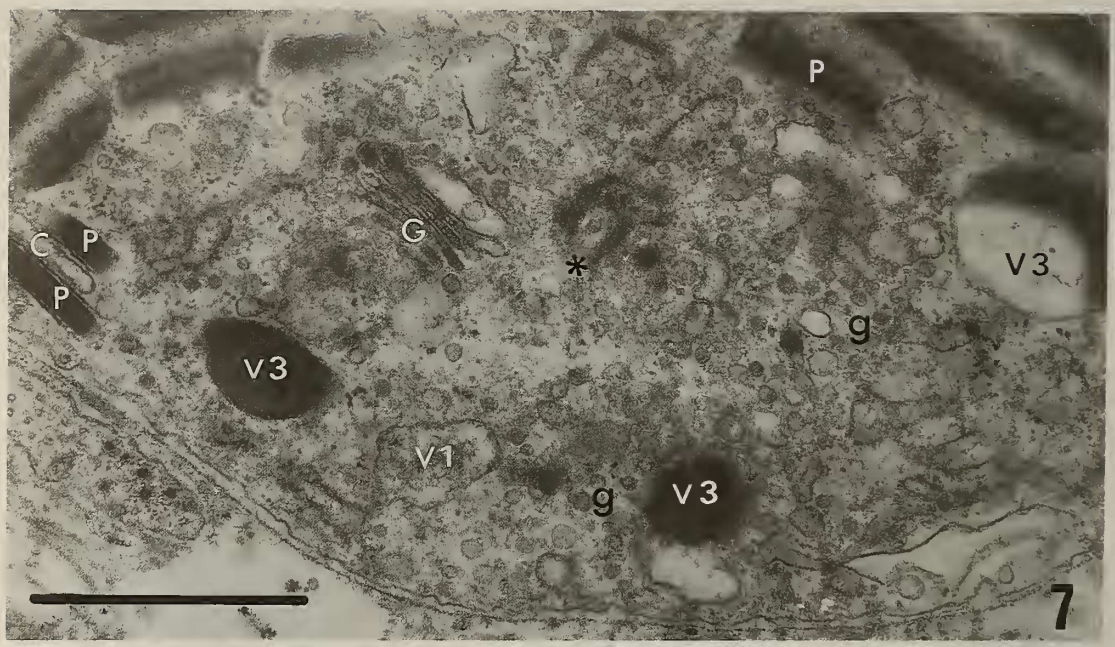


Fig. 5. Para-horizontal section through the platelets (P). Platelets are rectangular in shape and differ in size. They are arranged closely side by side to form a single plane of the reflecting surface. Bar indicates 1 μm. $\times$21,000

Fig. 6. Higher maginification of reflecting platelets in transverse profiles. Each platelet (P) is enveloped with a membrane (E). The envelope is separated from the inner platelet mass by 3 nm. The platelet shows fine substructure of 7 nm lattice. The cisterns (C) with 60 nm width are observed between neighbouring platelets. Bar indicates 0.1 μm. $\times$150,000.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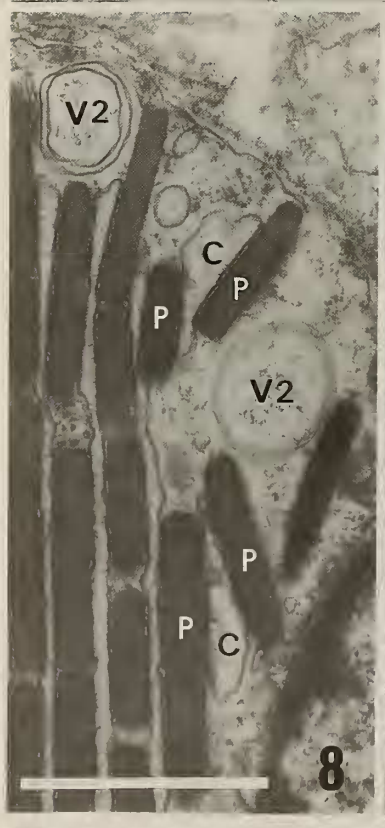
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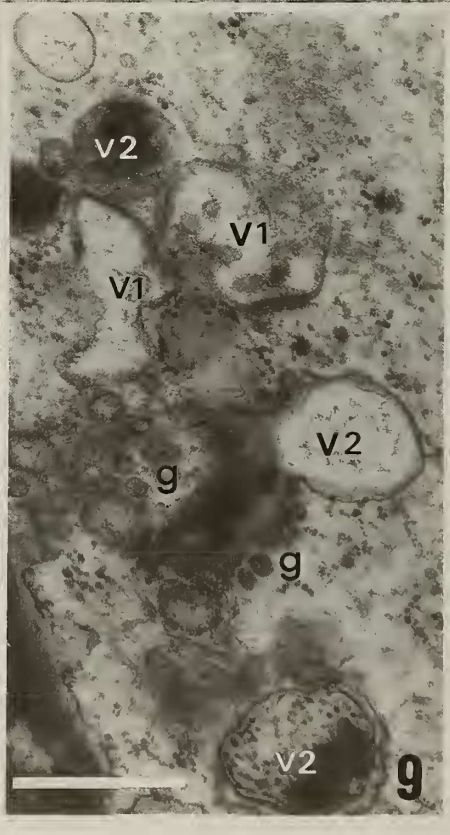
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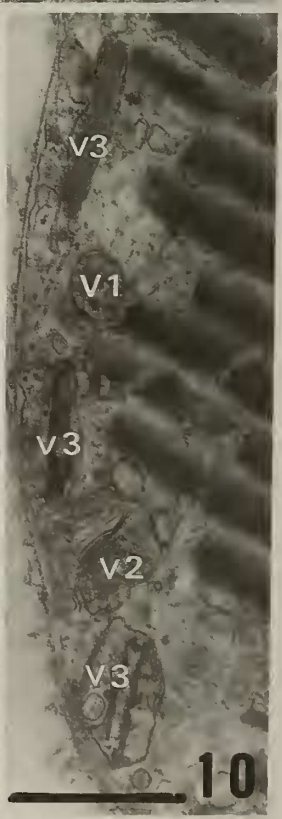
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V3
10

In the platelet forming area, three types of vacuoles were observed (Figs. 7~12). The first type of vacuole (marked as V1 in Figs. 7, 9 and 10) was irregular in shape and resembled distended rough surfaced endoplasmic reticulum. Some of these vacuoles contained various cytoplasmic components, such as vesicles and ribosome-like granules. The second type of vacuole (marked as V2 in micrographs) were round in profile and contained fluffy materials in the lumen. The second type of vacuole had a complex internal membrane structure and often appeared as a double walled vacuole (Figs. 8, 10~12). The last type (marked as V3 in micrographs) often had an elipsoidal or even elongated shape and contained a dense amorphous substance which was similar in appearance to the internal mass of the reflecting platelet.

A cluster of vesicles or smaller cisterns (marked as Vs in Figs. 3, 4 and 13) were observed along the developing platelets. These vesicles were aligned in a line at the place of the cistern between neighbouring platelets. Newly formed platelets were often seen in close contact, because of the absence of an intervening cistern (Fig. 12). Golgi bodies were often found in the platelet forming area in iridoblasts (Fig. 7). The outer lamella of the Golgi stack was distended in these cells. Golgi vesicles were also seen associated with the vacuoles or the devoloping platelets (Figs. 9 and 11). Some of the Golgi vesicles were seen directly fusing with the limiting membrane of the developing platelet (Fig. 11). The lumina of the Golgi lamellae and vesicles were filled with opaque material. Centrioles were often detected in close association with the Golgi complex. Microtubules were seen radiating from the centriole.

DISCUSSION

The overall appearance of the clam iridophores resembled those in cephalopods [2] and vertebrates [4, 8, 18]. The cytoplasm of the cell is fully occupied with tightly packed reflecting platelets. Platelets are aligned in rows and form multiple reflecting planes at each interface with the cytoplasm. Each platelet is bound with a membrane: the envelope. The membrane measures 7 nm in thickness and its dimensions are virtually identical to those of the plasma or cisternal membrane. The platelet is rectangular in shape and it has uniform thickness in each cell. The dimension, however, differs from cell to cell, ranging from 80 nm to 120 nm. The reflecting body of the platelet is electron dense and has a fine lattice that resembles the paracrystalline structure of proteins.

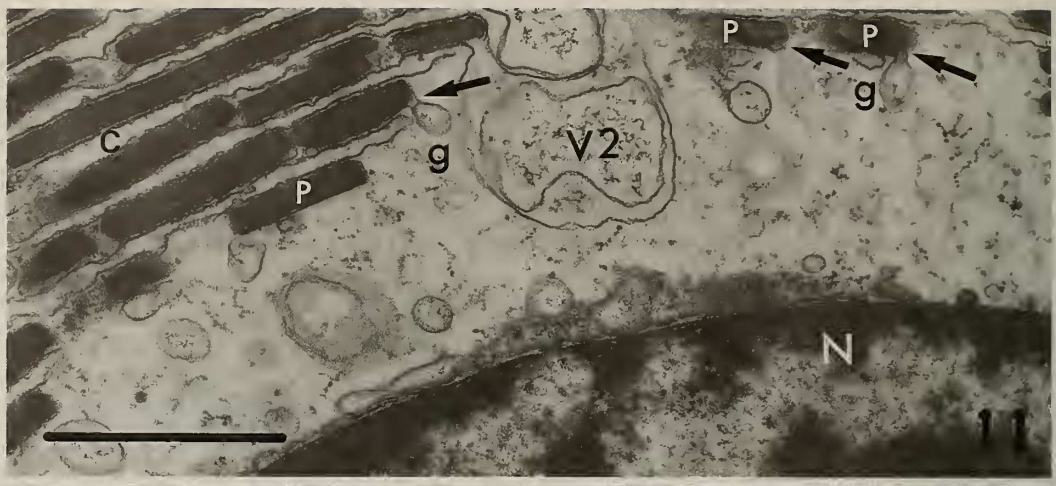
There are long and flat cisterns tightly secured between the rows of membrane bound platelets. Since the cistern is fairly uniform in width measuring 50 nm to 60 nm, the space between platelets is also kept in uniform. The intervening cistern illustrates the mechanism that secures the interplatelet space at a definite distance which is required for the efficient coloration of the iridophores [18]. In this respect, the clam iridophores differ from the cephalopod iridophores, in which the space between the platelets is the

Fig. 7. The platelet forming area near the cell periphery of a developing iridoblast. Diverse forms of vasuloes (V1 and V3) are seen around the Golgi body (G) and the centriole (*). Golgi vesicles (g) are seen associated with vacuoles. Some of vacuoles (V3) show the same internal density with the developing platelelts (P). A small cistern (c) is observed between developing platelets on the left of the micrograph. Bar indicates 1 μm. ×35,800.

Fig. 8. Platelet forming area where some of vacuoles are seen double walled (V2). Fluffy material in the vacuoles appears similar to the cytoplasmic matrices. Developing cistern (C) formed by fusion of vesicles are seen between two developing platelets (P). Bar indicates 1 μm. ×32,000

Fig. 9. Another platelet forming area showing condensation of dense material in the vacuole (V1 and V2). Vesicles, ribosome-like granules and fluffy materials are seen in the lumina of the vacuoles. Some vacuoles (V2) are double walled. Golgi vesicles (g) are seen accumulating around the vacuole with dense materials. Bar indicates 0.5 μm. ×45,000

Fig. 10. Vacuoles showing transitinal stages of the platelet formation. Condensation process of the dense materials is seen in the developing platelets (marked V1, V2, and V3, which indicate the transitional vacuoles in the numerical order). Vacuole at the final stage (V3) sppears almost similer to the platelet, except its loose envelope and less dense internal mass. Cytoplasmic components are seen inside of all vacuoles. Transverse sections through the dense mass of the V3 vacuoles are shown as V3s in Figs. 7 and 13. Bar indicates 1 μm. ×23,000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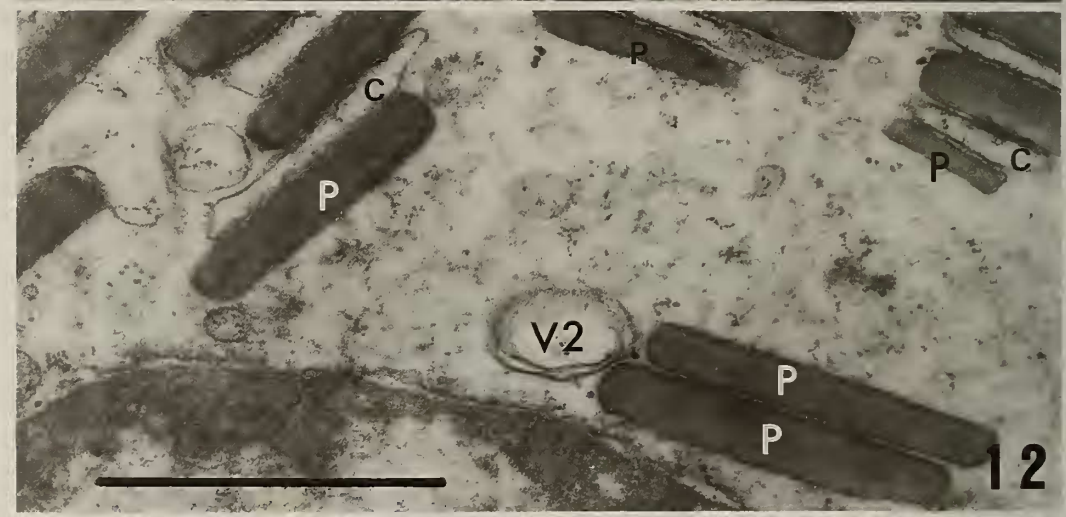
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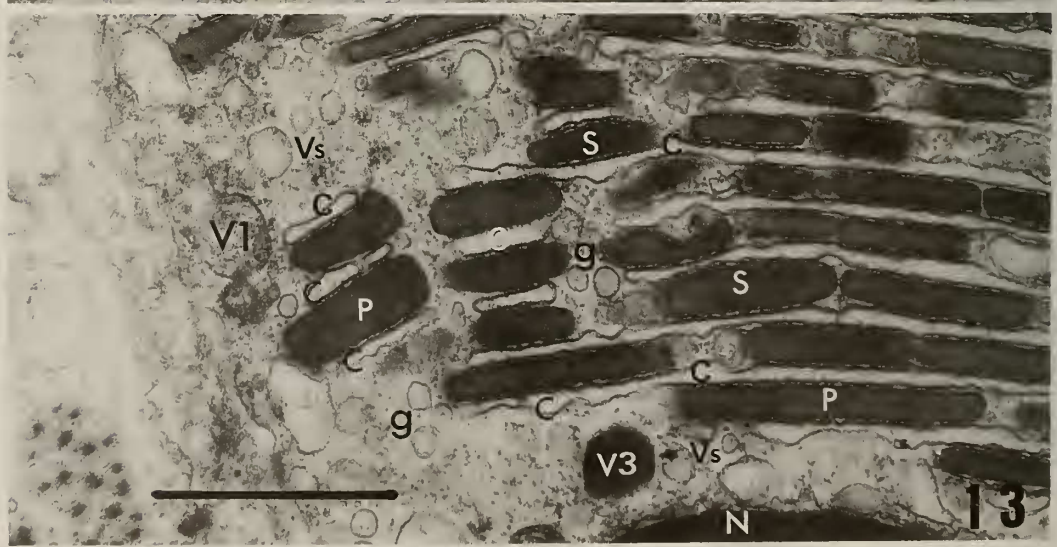
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extracellular, so that the platelet is not bound to membrane and is free in the cytoplasm [3, 9, 6, 10].

As well as the structural organization, the physical or chemical nature of the platelet may influence the coloration of the cell. Clam iridophores are sectioned smoothly on the glass knives, suggesting pliablity of the platelet element, while purine (such as guanine) platelets in vertebrate iridophores are brittle, so that well sectioned profiles for electron microscopy are not easily obtainable. Thus, purines do not seem to be the major component of platelets in the clam iridophore. The platelets in cephalopod iridophores are also readily sectioned and appeared similar to those of clam iridophores under the electron microscope [2, 3, 7]. There have been diverse reports on the nature of platelets in cephalopod iridophores, such as guanine [3], purines [7], chitin [11] or protein [6, 9]. The paracrystalline structure with fine lattice observed in this study may indicate that the platelet in clam iridophores is mainly of proteineous rather than purine nature, although the latter cannot totally be excluded.

Clam iridophores display various colorations ranging from blue to yellow-green, or sometimes even to red, depending on their distribution. This indicates that the thickness and/or arrangement of reflecting platelets differ accordinng to the cell. As mentioned previously, the thickness differs from cell to cell ranging from 80 nm to 120 nm, although it is fairly uniform within a single cell. Providing that the optical path of the reflecting layer in efficient iridophores equals a quarter wavelength of the reflected spectrum [12, 18], the platelet in clam iridophores which has blue to red interference colors and a thickness ranging from 80 nm to 120 nm seems to have a refractive index (n) of around 1.5. This is almost the same value as that of chitin [11].

In developing iridoblast, platelets are formed in a confined area around the nucleus. In this platelet forming area various organelles, such as endoplasmic reticula, vesicles and vacuoles are found. The vacuole seems to be derived from the endoplasmic reticulum and finally become a platelet envelope, because various forms that suggest a gradual transition from the distended endoplasmic reticulum (V1) to the envelope (V3) are observed in the area. Vacuoles containing vesicles and ribosome granules (V1) are morphologically similar to the "vesiculo-globular bodies" or the "multivesicular bodies" observed in developing melanoblasts in mouse skin [13] or goldfish fin [14], respectively. These vacuoles seem to be in the earlier stages of the platelet formation in the giant clam iridophores and are considered to be equivalent forms to the primordial vesicle proposed by Bagnara [15, 16] for the common precursor to all pigment organelles in vertebrate chromatophores. Double walled vacuoles are frequently seen in the platelet forming area (V2 in Figs. 9, 11 and 12). The similar structure in vertebrate iridoblasts (double walled saccule) is shown to be formed by an invagination or infolding of a vacuole (7). However, this is not demonstrated in the clam iridoblasts.

The accumulation process of the dense reflecting material is also shown in transitional internal structures of the vacuoles. Since the lumina of the earlier vacuoles are slightly denser than the cytoplasm, the accumulation of the reflecting material in these vacuoles seems to have a low concentration (V1 and V2). In the later stage of the vacule, dense materials appear at the middle of the vacuole and are gradually condensed into a rec-

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Fig. 11. A portion of the platelet forming area. No cistern are observed along th developing platelets (P) at the margin of the row. Golgi vesicles are seen fusing with the limiting membrane of the developing platelets (arrows). Double walled vacuole (V2) with fluffy material in the lumen is seen close to the developing platelets. Bar indicated 1 μm. $\times$32,500

Fig. 12. Newly formed platelets (P) which are not yet arranged into a row are seen near the nucleus. These platelets show clear limiting membrane, but have no intervening cistern, so that they are closely contacted each other. Bar indicates 1 μm. $\times$46,000

Fig. 13. Platelet forming area where developing platelets (P) dispose at the margin of the platelet row. A series of vesicles (Vs) are seen at the position of the cistern. Newly formed cisterns (c) which still appear as flattened vesicles are positioned both sides of the developing platelets. Developing platelets show transitional forms from spherical one (V3), to spindle (S), or rectanglar (P) one. Bar indicates 1 μm. $\times$32,000

tagular platelet mass (Fig. 11, V3). When the vacuole in this stage is sectioned through the dense accumulation at the middle, it appears as a dark vacuole (Fig. 7). The incorporation of Golgi vesicles onto the developing platelets is frequently observed (Figs. 7 and 11). The involvement of the Golgi vesicles may indicate the possibility of protein and/or carbohydrate (chitinous) as components of the reflecting platelet in the giant clam.

The intervening cisterns are formed from vesicles (Vs in Figs. 3 and 4). These vesicles are also dilated, but are somewhat smaller than the vacuoles involved in the platelet formation. Dilated vesicles are seen to be depressed between small developing platelets which are not yet assembled in rows (Figs. 8 and 13). These vesicles are opaque in appearance and contain flocculent matters in the lumina. They appear in a line between developing platelets and seem to fuse with each other to form a long and flat cistern (Figs. 3 and 4).

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